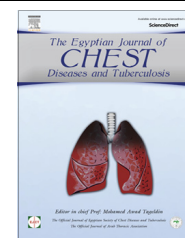




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ORIGINAL ARTICLE

Receptor of advanced glycation end products in childhood asthma exacerbation



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KEYWORDS

Asthma;
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Abstract *Background:* Receptor of advanced glycation end products (RAGE) is a new marker of inflammation in different inflammatory disorders. Bronchial asthma as an inflammatory disease is associated with increased levels of many markers of inflammation which are used to evaluate the severity of childhood asthma, and may be the targets of new therapeutic options aiming at improving the management of such diseases.

Aim: To evaluate serum levels of RAGE in acute asthma exacerbation in children, and also to correlate their levels with peak expiratory flow rate (PEFR) and number of exacerbations during the last 6 months.

Methods: One hundred asthmatic children, aged 6–12 years, with acute exacerbation were evaluated during their visit to ER unit for serum RAGE (measured by ELISA) and PEFR before receiving any treatment. They were 53 males and 47 females. Fifty subjects were taken as controls for serum RAGE.

Results: Serum RAGE was significantly higher in asthmatic children compared to control group. It was higher in asthmatic children who had poor response to initial therapy at emergency room. Serum RAGE showed significant negative correlation with PEFR and significant positive correlation with a number of asthma exacerbations during the last 6 months.

Conclusion: Serum RAGE is elevated during acute asthma exacerbation in children. High levels above 1733.5 pg/mL can expect poor response to initial therapy at emergency room and need for hospitalization with sensitivity and specificity and a specificity of 90.5% and 83.35% respectively.

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Introduction

Bronchial asthma is by far the most common chronic disease of childhood and it is considered to be the leading cause of childhood morbidity caused by chronic diseases leading to

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absence from school, emergency department (ER) visits and hospitalization [1].

Asthma exacerbation is a well known factor that can increase the risk of asthma mortality [2].

As an inflammatory disease, pediatric asthma was associated with high levels of many inflammatory markers [3,4].

The receptor for advanced glycation end products (RAGE), a member of the immunoglobulin superfamily of cell surface molecules, is involved in the signal transduction from pathogen substrates to cell activation during the onset and continuation of inflammation [5].

Its involvement in inflammation has been suggested, that RAGE is upregulated in all inflammatory lesions studied, including rheumatoid arthritis, inflammatory kidney disease, arteriosclerosis, inflammatory bowel disease and others [6].

RAGE was first identified in lung tissue, it is located on the basolateral membranes of alveolar epithelial type I cells but RAGE mRNA has also been found in alveolar epithelial type II cells [7].

The aim of this study was to evaluate the serum levels of RAGE in asthmatic children during exacerbation compared to control subjects and find a relationship between these levels and outcome of asthma exacerbation.

Subjects and methods

This study was carried out in Pediatric Department, Tanta University Hospital, Egypt between June 2013 and December 2013.

We examined 100 children attending the emergency room of Pediatric Department with asthma exacerbations. A control group consisting of fifty age and sex matched children was included in our study.

Written informed consents were obtained from parents of all children.

Inclusion criteria of patients:

- Age was ranging from 6 to 12 years.
- Diagnosis of bronchial asthma according to GINA guideline and National Asthma Education and Prevention Program, 2007.
- Patients were coming to emergency room due to exacerbation of their asthma.

Exclusion criteria:

- Patients with other pulmonary disorders as bronchiectasis and cystic fibrosis.
- Children below 6 years or above 12 years of age.
- Children of control group had no chest troubles and negative history of steroid use in last 6 months. All of them had negative personal and family history of wheezy chest, asthma or nebulizer use before.

All subjects had detailed history taking, and thorough clinical examination. Peak expiratory flow rate (PEFR) was measured in all of them.

Blood samples were taken from all patients in the ER unit during acute asthma exacerbation before any rescue medication was given to them.

Patients were given standard treatment of asthma exacerbation and then re-assessed after 1 h for response to initial therapy. Children with good response are who did not need admission and with no return of exacerbation for 1 week. Children with poor response are who needed admission to the hospital or returned back within 1 week with another exacerbation.

Determination of RAGE serum level: estimation of RAGE in the serum pg/mL by enzyme immunoassay supplied by Ray-Biotech, Inc.

The RayBio® Human RAGE ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human RAGE in serum. This assay employs an antibody specific for human RAGE coated on a 96-well plate. Standards and samples are pipetted into the wells and RAGE present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human RAGE antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of RAGE bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Results

One hundred children with asthma exacerbation and fifty control children were included in our study. The demographic data and peak expiratory flow rate (PEFR) of both patients and control are demonstrated in Table 1.

Mean RAGE serum levels were 1770.1 ± 423.3 pg/mL in children with asthma exacerbation, and 745 ± 224.6 pg/mL in control children (Table 2).

Serum RAGE levels showed significant negative correlation with PEFR and significant positive correlation with a number of asthma exacerbations during the last 6 months before inclusion in the study (Table 3).

After initial therapy in the emergency room, 55 patients showed good response and 45 patients showed poor response.

Table 1 Demographic data and peak expiratory flow rate (PEFR) of the studied subjects.

	Control (No = 50)	Asthmatic (No = 100)	<i>t</i>	<i>p</i>
Age in years	8.1 ± 1.8	8.2 ± 2.1	1.1	0.28
BMI in kg/m ²	20.8 ± 3.8	21.3 ± 4.2	1.2	0.25
Male/female	24/26	53/47	$\chi^2 = 1.6$	0.48
Age at time of diagnosis	–	3.2 ± 1.6		
Associated nasal allergy	–	55%		
Immediate family history of asthma	6%	58%		
Blood eosinophils %	2.1 ± 0.3	7.1 ± 2.4	5.6	0.009*
PEFR (% of predicted)	93.1 ± 8.3	48.2 ± 6.4	6.3	0.008*

* Significant.

Table 2 Comparison between cases and control according to their sRAGE.

	RAGE	
	Patients	Control
Range	855–2534	121–946
Mean	1770.1	745
±SD	423.3	224.6
<i>t</i> -test	48.225	
<i>p</i> -value	0.001*	

* Significant.

Table 3 Correlation between serum RAGE and both PEFR and the number of asthma exacerbations during the last 6 months.

Correlation between sRAGE and:	<i>R</i>	<i>p</i>
PEFR	−0.9	0.011*
No of exacerbations during the last 6 months	0.992	0.013*

* Significant.

On reviewing the sRAGE levels of both of them it was noticed that it was significantly higher in those with poor response than those with good response (1920 ± 432 versus 1540 ± 390 pg/mL respectively with $t = 6.5$ and $p = 0.007$).

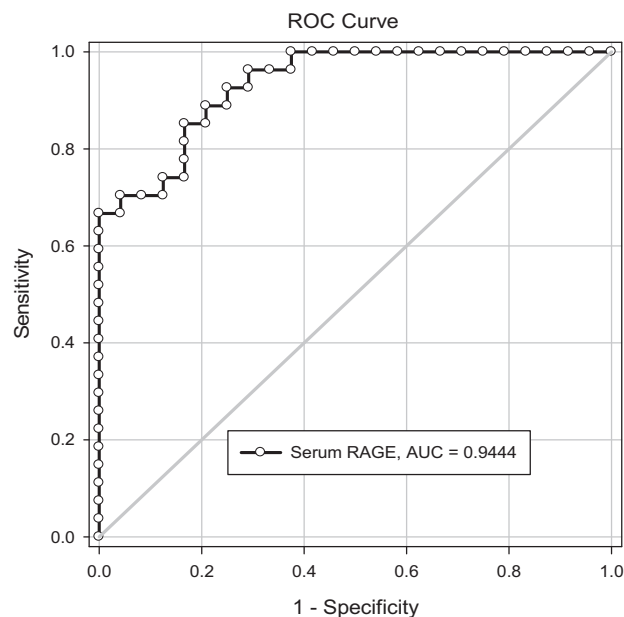
Receiver-operating characteristic (ROC) curve analysis showed that serum RAGE level had a sensitivity of 90.5% (95% confidence interval (CI): 69.27–98.81%) and a specificity of 83.35% (95% CI: 62.6–95.3%) for differentiating between good and poor response to initial therapy at emergency room at an optimal cutoff value of 1733.5 pg/mL. The likelihood ratio positive (LR+) was 5.412, and the likelihood ratio negative (LR−) was 0.114. The area under the ROC curve (AUC) was 0.9444 (Fig. 1).

Discussion

Historically, the receptor for advanced glycation end products (RAGE) was thought to exclusively play an important role under hyperglycemic conditions. However, more and more evidence suggests that RAGE in fact is an inflammation perpetuating multi-ligand receptor and participates actively in various vascular and inflammatory diseases even in normoglycemic conditions [8].

Under pathologic conditions, RAGE is up-regulated in blood vessels, neurons, and transformed epithelia and is involved in several chronic diseases, such as rheumatoid arthritis, diabetes, inflammatory kidney disease, arteriosclerosis, inflammatory bowel disease, neurodegenerative disorders, and wound-healing disorders [9].

Most tissues display a relatively low expression of RAGE in their normal physiological state. However, lung has been identified as an exception; as RAGE is constitutively expressed at a high basal level in pulmonary tissue, localized primarily in alveolar type I (ATI) pneumocytes [10]. Alterations in RAGE levels and RAGE–ligand interaction have been suggested to play a relevant role in the pathogenesis of several pulmonary diseases. Acute lung injury (ALI) and acute respiratory distress

**Figure 1** ROC curve showing serum RAGE to differentiate between good and poor responder to initial ER therapy with sensitivity and specificity 90.5% and a specificity of 83.35% respectively at an optimal cutoff value of 1733.5 pg/mL.

syndrome (ARDS), a more severe manifestation, are syndromes of acute respiratory failure, hallmarked by hypoxemia, disruption of alveolar fluid clearance (AFC), deterioration of the alveolar-capillary barrier, and, most importantly, damage to ATI pneumocytes [11,12].

Increased levels of RAGE were demonstrated in bronchoalveolar lavage fluid (BALF) in various direct models of lung injury induced separately by intratracheal instillation of hydrochloric acid, lipopolysaccharide (LPS), or *Escherichia coli* as well as exposure to hyperoxia [13].

In the present study, we evaluated the serum level of RAGE in asthmatic children during asthma exacerbation before receiving any rescue treatment in ER in 100 asthmatic children and 50 healthy controls.

Up to our knowledge, there are no published papers evaluating serum levels of RAGE in asthmatic children during exacerbation in Middle East, reflecting the importance of this study.

We found the mean serum levels of RAGE were higher in asthmatic patients during exacerbation compared to control group, indicating that RAGE may be considered as an inflammatory marker sharing in the pathogenesis of childhood asthma, and it may be the target of new therapeutic interventions. Moreover, we found that serum RAGE was even higher in children who showed poor response to initial therapy at the emergency room reflecting its importance in evaluating drug response in asthmatic children. Serum RAGE was able to differentiate between good and poor responder to initial ER therapy with sensitivity and specificity 90.5% and a specificity of 83.35% respectively at an optimal cutoff value of 1733.5 pg/mL.

Also, sRAGE showed a significant negative correlation with PEFR and significant positive correlation with the number of asthma exacerbations during the last 6 months.

Our results are in concordance with those of Iwamoto et al. [14] who found that blood level of RAGE was found to be higher in asthmatic subjects in comparison to COPD patients and control group. Another experimental study [15] has shown that RAGE is more expressed in asthmatic airways and giving RAGE inhibitors markedly decreased inflammation in animal model of asthma. El-Seify et al. [16] found that Serum level of sRAGE correlated with severity of bronchial asthma clinically and functionally.

More multicenter studies are needed on larger number of asthmatic children and to detect also the effect of anti-asthma medications on serum RAGE.

Conclusion

Serum RAGE is elevated during acute childhood asthma exacerbation in comparison to control subjects. High levels above 1733.5 pg/mL can expect poor response to initial therapy at emergency room and need for hospitalization with sensitivity and specificity 90.5% and a specificity of 83.35%.

Conflict of interest

Authors declare that they don't have any conflict of interest.

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